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Ageing as a determinant of renal and vascular disease: role of endothelial factors

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Keywords: endothelium; growth; hypertension; hypertrophy; inflammation; reversibility

Introduction

In developed countries, ageing is the most important risk factor for age and death after age 28. Age also determines the onset and development of the most prominent vascular and renal diseases, atherosclerosis and glomerulosclerosis. Increased vascular and renal oxidative stress, and, as a consequence, abnormal activity of endothelium-derived molecules, such as nitric oxide (NO), angiotensin II and endothelin, are now recognized as important mechanisms controlling these disease processes. In this article, I will discuss current evidence for the involvement of endothelial factors in the genesis of vascular dysfunction and cardiorenal disease seen with ageing and present therapeutic approaches to actively interfere with these disease processes.

> 'Aging changes can be attributed to development, genetic defecects, the environment, disease, and the inborn aging process
>
> The latter is the major risk factor for disease and death after age 28 in the developed countries'.
>
> Denham Harman [1].

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Ageing and development of cardiorenal diseases

The majority of deaths worldwide in the year 2020 will be due to cardiovascular causes, and a substantial proportion of this number will be due to the increase in the aged population expected in the next two decades [2,3] Moreover, ageing will continue to be the most important determinant of disease in Western societies [1]. Ageing not only promotes the development of vascular disease and glomerulosclerosis [4], but is also associated with significant metabolic changes, resulting in age-dependent increases of the body mass index, development of insulin resistance and/or diabetes, as well as changes in lipid metabolism [5–9]. The incidence of hypertension increases in the elderly and may be related to enhanced sodium sensitivity and activation of the sympathetic nervous system (which are also charactaristic features of chronic renal failure [10]), as well as abnormal responses to certain drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) [11]. Since all the changes described above may contribute to atherogenesis, one could argue that the increase in renal and vascular disease seen with ageing could be simply explained by these disturbances. However, the pathogenesis of age-dependent diseases appears to be more complex since it also involves local cellular changes in the kidney, the vasculature and circulating blood cells (reviewed in [12]).

Cell injury precedes onset and determines progression of disease

Endothelial cells form the inner lining of arterial blood vessels and amount to ~1.5 kg, covering an area of approximately four tennis courts [13]. Under healthy conditions, endothelial cells constantly produce a number of vasoactive and trophic substances that control inflammation, vascular growth, vasomotion, platelet function, and plasmatic coagulation. Among others, these substances include prostacyclin, NO, superoxide anion (O_2^-), angiotensin II, as well as endothelin-1 (reviewed in [13,14]). If disease—or physiological processes such as ageing or menopause—sets in, endothelial cell function deteriorates, and the finely tuned release of growth inhibitors and mitogens becomes dysbalanced (Figure 1).

In the vasculature, early lesions of the atherosclerotic plaque (fatty streaks) consisting of endothelial deposits of lipid-laden macrophages [15] can be detected in the fetal aorta, and their progression is aggravated by maternal hypercholesterolaemia [16]. This suggests that lipids play an essential role for disease onset and progression of atherosclerosis already early in life. Vascular endothelial cell injury is a key event in atherogenesis [15], indicating that under normal conditions intact endothelial cells protect from atherosclerosis. Similarly, in the kidney, damage of glomerular endothelial cells has been reported to contribute substantially to sclerosis of the glomerulus [12]. Consequently, endothelial factors such as endothelin-1 have been identified to play a direct role in the genesis of experimental glomerulosclerosis and atherosclerosis (reviewed in [14]), and analogies between 'accelerated ageing' and uraemia have been proposed [17]. Thus, it would not be surprising if changes in production and/or activity of these mediators with ageing would either promote or delay the disease process. Some of the first direct evidence for this hypothesis will be discussed below.

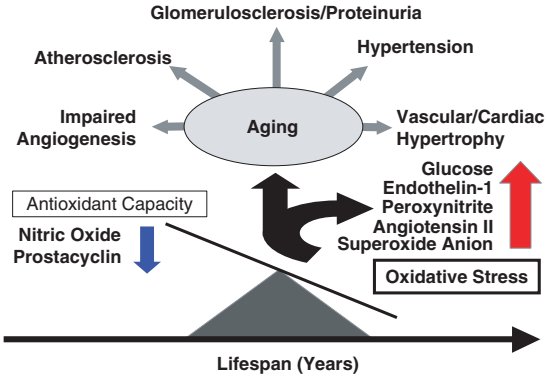

Fig. 1. Proposed mechanisms of the vascular and renal ageing process. A continous increase in cellular oxidative stress with ageing results in a shift promoting activity and production of vasoactive mediators. Enhanced formation of growth-promoting factors such as superoxide anion, angiotensin II and endothelin-1 counteracts the loss of anti-inflammatory and growth inhibitory mediators such as nitric oxide and prostacyclin with increasing age.

Importance of the L-arginine/nitric oxide pathway

NO, a short-lived gaseous molecule, is the most important endogenous vasodilator, which also shares strong anti-aggregatory and anti-inflammatory properties [18]. NO reacts with O_2^- at a diffusion-limited rate of 6.7×10^9/s, thereby reducing the bioactivity of NO and resulting in formation of the cytotoxic peroxynitrite [19]. In rats, a species normally resistant to atherosclerosis but not to glomerulosclerosis, ageing is associated with a marked decrease of basal [20] as well as stimulated endothelial NO bioactivity in the systemic arterial circulation [20,21]. Impaired function of endothelium-dependent pathways has also been observed in rat coronary arterioles [24], vessels which in humans do not develop atherosclerosis even if epicardial arteries are affected. An attenuation of endothelium-dependent vasodilatation with ageing has been observed in the human brachial artery [22].

The age-associated reduction of NO bioactivity is associated with an increase in expression of the 'inflammatory' isoform of NO synthase, NOS2 [23,24], increased NADPH oxidase activity and formation of O_2^- [24,25]. As a consequence, vascular peroxinitrite formation increases, causing nitrosylation and functional alteration of vascular proteins [19,24]. Indirect evidence suggests that alterations of the L-arginine/NO

pathway also occur with ageing. These observations include reductions of circulating NO metabolites [26] and changes in basal NO release [20,27], as well as reduced renal NO metabolite excretion [27]. While vascular NOS2 expression increases with ageing [23,24], NOS3 isoenzyme expression appears to be regulated depending on gender [20,23]. We have shown previously that in aged Wistar rats, aortic NOS3 gene expression decreases in females [20] while an increase occurs in males [23]. This finding has been confirmed recently by Pollock's group, who found a similar regulation pattern in aged rat mesenteric arteries [28]. With intermediate ageing, tissue levels of the stable NO metabolites, nitrate/nitrate, decrease in the kidney [29]. However, with advanced ageing, changes occur in an anatomically distinct pattern, showing decreased levels of NO metabolites selectively in the renal cortex but not in the medulla [30]. This selective regulation of NO bioactivity may be related to distinct local changes of factors regulating renal NO release, such as local increases in endothelin-3 [30,31].

Other endothelial factors, such as vascular endothelial growth factor (VEGF), also appear to be involved in age-dependent changes and are regulated in an NO-dependent fashion. There is experimental evidence to suggest that the ability for tissue repair through sprouting of new vessels (angiogenesis), which requires the presence of VEGF, is impaired in aged mice [32,33]. Plasmid-mediated gene transfer of DNA encoding human *VEGF165* can increase hindlimb angiogenesis in aged animals after 40 days comparably with the degree of vascularization seen in untreated young animals. Whether the expression of VEGF and its therapeutic effects on angiogenesis can also be achieved thereafter remains uncertain. Also, given the safety concerns that arose from human VEGF gene therapy trials, it is unlikely that gene therapy represents an option to interfere successfully with vascular ageing in humans.

Age-associated changes due to endothelial factors: truly irreversible?

Over the past centuries, scientists have developed more than 300 theories to explain the ageing phenomenon, many of which are based on the notion that age-dependent changes accumulate with time [34]. The 'free radical theory of ageing' was put forward by Denham Harman already half a century ago (reviewed in [34,35]) and is based on the chemical nature and ubiquitous presence of free radicals. Indeed, several lines of evidence now indicate that cellular oxidative stress caused by reactive oxygen species (ROS) is an important factor contributing to ageing-associated organ injury. In addition to ROS-induced DNA damage in the nucleus as well as in mitochondria, ageing of endothelial cells is associated with an 'inflammatory' phenotype as well as alterations of cell organelles, signs of cell senescence, abnormal activity of cellular mediators and/or enzymes and vascular reactivity of vascular smooth muscle cells [23,29,36–39]. At the level of the endothelial cell, oxidative stress causes cellular damage by oxidative modification of expression and function of proteins [37–39]. Therefore, it would be desirable that any treatment aiming at interfering with or even restoring abnormal age-dependent function or structure should, at some point, inhibit the production and/or activity of ROS or enzymes involved in ROS production. There is evidence suggesting that in certain forms of disease, ROS inhibition favourably affects outcome. Indeed, blockade of endothelin receptors in experimental diabetes recently has been shown to inhibit expression of the NAPDH oxidase subunit $p22_{phox}$, an important source of vascular ROS [40,41], and similar data have been obtained with angiotensin AT_1 receptor blockers (ARBs) [42]. Thus, is it not surprising that angiotensin-converting enzyme inhibitors (ACEIs) and ARBs as well as endothelin receptor blockers are effective in preventing experimental age-related functional changes of arterial endothelial cells [43–45].

Experimental studies indicate that endothelium-dependent relaxant responses to acetylcholine are markedly reduced in the aged rat aorta, whereas the response is maintained in certain vessels such as the femoral [20] or the mesenteric artery [28]. A similar heterogeneity has been described with regard to expression of cyclooxygenase isoenzymes between ageing rat aorta and superior mesenteric artery [46]. These experimental data suggest that not only mediator activity but also transcriptional regulation of enzymes regionally differ within the ageing vasculature. If applicable for the human situation, this could at least in part explain the heterogeneity of susceptibility to atherosclerosis. Indeed, certain arteries such as the internal mammary artery and the radial artery rarely develop atherosclerosis even up to high age. Vascular activity of the antioxidant enzyme superoxide dismutase in rats is not altered by ageing [20].

In addition to the impaired NO bioactivity, which would promote vasoconstriction, there are also increases in vascular reactivity to vasoconstricting substances such as angiotensin II, endothelin-1 [29], vasoconstrictor prostanoids like prostaglandin H_2/thromboxane A_2, and enzyme expression of prostaglandin H synthase [46,47]. It has been shown by Remuzzi and co-workers that inhibiting the activity of angiotensin II slows the development of age-dependent glomerulosclerosis in conjuction with blood pressure lowering and a reduction of tubulointerstitial injury [49]. One of the most potent endothelial factors, endothelin-1, not only directly impairs vasomotion [48], but also controls pathological processes related to ageing. Based on our previous observation that ageing increases renal endothelin expression in the absence of hypertension [23,29], we recently have addressed the question of whether this activation might contribute to the pathogenesis of spontaneous glomerulosclerosis in the ageing kidney. Unexpectedly, we found that short-term inhibition of endothelin ET_A receptors using darusentan reversed established glomerulosclerosis and proteinuria

in aged rodents, effects that were associated with partial restoration of podocyte structure [50]. Interestingly, restoration of glomeruli was associated with decreased expression of $p21^{WAF/CIP1}$ and matrix metalloproteinase-9, and independent of tubulo-interstitial injury, blood pressure, creatinine clearance or renal blood flow [50]. This indicates that ageing adversely affects the structure of the renal glomerulus in a locally confined and reversible manner, involving ET_A receptor-mediated mechanisms that are also involved in the regulation of age-dependent changes in electrolyte excretion [51].

Therapeutic approaches

There are several options to improve vascular function in the ageing vasculature. In addition to the experimental data using gene transfer, there are complementary approaches for 'therapeutic' angiogenesis and maintaining vascular function such as exercise training and certain cardiovasculas drugs. In healthy animals, angiogenesis was increased in trained rodents as compared with sedentary animals, and the beneficial effect was abrogated by an anti-VEGF antibody [52]. A recent study in a transgenic mouse model of ageing also suggests that age-dependent reduction of angiogenesis can be effectively prevented by use of statin therapy [53]. Physical training in humans also helps to counteract the impairment of endothelium-mediated vasodilatory capacity normally seen with ageing [22,54–56], also suggested by a study comparing untrained with trained elderly men above 70 years of age [57]. Interestingly, exercise in the lower extremities may affect endothelial vasomotion in remote organs such as the arm [58], suggesting that physical exercise has systemic and possibly sustained benefical effects.

As outlined in Figure 2, several modalities are available to interfere with age-related changes in endothelial cell function. Preventive measures, which are often already applicable for juveniles, include cessation of smoking, reduction of increased body weight, and avoiding unbalanced diets rich in fat and sugars and low in fibres. Interestingly, nutritional additives such as vitamins appear to be largely ineffective in interfering with age-dependent functional changes. As ageing is frequently associated with a reduction of physical activity and fitness [59], it is even more important to emphasize the 'therapeutic' role of regular physical activity, which also helps to reduce the incidence and severity of related co-morbidities such as diabetes, high blood pressure, dyslipidaemia and obesity [60]. Finally, cardiovascular health awareness must be increased not only in the elderly but also in parents, including regular check-ups with their primary care physician, awareness of risk factors/blood cholesterol levels, and blood pressure measurements. If these changes in lifestyle have been implemented and are still insufficient, medical therapy aiming at improving vascular and renal homeostasis, and reversibly restoring organ dysfunction, such as cardiac hypertrophy or proteinuria, using statins, inhibitors of the renin–angiotensin–aldosterone system (RAAS), or possibly the endothelin system can be initiated. It can be anticipated that maintaining or even improving cardiorenal health with age is not only likely to result in improved general health but can also be expected to have have a positive impact on cardiovascular and renal morbidity and mortality [61–63].

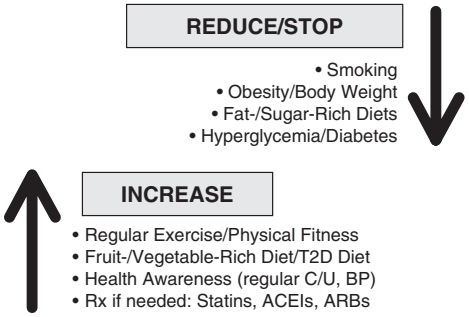

Fig. 2. The ageing endothelium: therapeutic options. Modalities to improve/reduce oxidative injury to the vascular wall and vascular endothelial cells in young as well as in elderly individuals. T2D = type 2 diabetes; C/U = medical check-up; BP = blood pressure; Rx = prescription; ACEI = angiotensin converting-enzyme inhibitor; ARB = angiotensin AT_1 receptor blocker

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Conflict of interest statement. None declared.

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Why patients with progressing kidney disease are referred late to the nephrologist: on causes and proposals for improvement

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Keywords: chronic kidney disease; dialysis; end-stage renal failure; improvement of patient care; late referral

The adverse effects arising from late referral (LR) have been reported by nephrologists over the past 20 years from several countries [1–10]: not only does LR delay the introduction of measures to attenuate the progressive loss of kidney function and prevent uraemic complications [11], but LR has also numerous short and long-term deleterious effects on clinical outcome [1–8]. The only study that did not confirm the long-term harmful effects of LR is the study of Roubicek *et al*. [12]. It appears, however, that their definition of LR was longer (4 months before dialysis), patients were younger, with less co-morbidities and relatively long hospitalization times in both patient groups, and a shorter mean survival time of the early referral group than in most other patient series.

While a recent review analyzed the relationships between LR, mortality and morbidity, and the potential positive effects of early referral [10], the present editorial comment identifies and analyzes the different causes responsible for LR and suggests some

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